Anatomy and functional status of haustoria in field grown sandalwood tree (*Santalum album* L.)

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A study on the physiology of root parasitism in sandal (*Santalum album* L.) was conducted by comparing a six-year-old sandal grown alone and along with a host (*Casuarina equisetifolia*). Although maximum haustorial connections were observed when grown along with the host, sandal formed haustorial connections with plants including grass up to a distance of 3 m. Anatomical studies on haustoria indicated a vascular connectivity between the host and sandal. While the haustoria functioned as a physiological unit supporting nutritional requirements of sandal, direct lumen–lumen xylem connections between sandal and host were absent. Functional status of the haustorial connection was studied by radio-labelling the host (*Casuarina*) and nearby grass with phosphorus (^{32}P). Presence of translocated ^{32}P in sandal was noticed after six hours of labelling the host. ^{32}P activity was noticed from eight days up to 16 days after which then it started decreasing. Study with multiple hosts revealed that the extent of translocation from hosts to sandal varied from 28.9% (coconut + *Casuarina* + rubber as host) to 78.5% (*Casuarina* + rubber as hosts). Reverse translocation of ^{32}P from sandal to host was also observed. The study concludes that it is not necessary to plant the host along with the sandal as it is practiced presently.

Keywords: Haustoria, radio tracer, root parasitism, sandal.

SANDAL tree (*Santalum album* L.) is a precious tree well known for its fragrant wood (East Indian Sandalwood) and the scented oil derived from it (East Indian Sandal tree oil). Sandal is a member of the family Santalaceae and locally called *chandan*. *S. album* is indigenous to India covering an area of 9600 sq. km (ref. 1) with most of the area (90%) falling in Karnataka and Tamil Nadu[2]. The annual production of sandalwood in India has been declining. Data shows that production declined from 4000 MT (metric tonne) per year in the 1950s to 500 MT in 2007 (ref. 1). The global annual demand for sandalwood is estimated to be about 5000–6000 MT wood and 100–120 Mg oil. The major issues related to depletion of sandal in forests include illegal feelings, smuggling and diseases[3].

Due to the widening gap between demand and supply, sandal has a great potential as plantation, and as component of agro-forests. However, large scale planting of sandal has not picked up due to poor establishment, resulting from its root parasitic nature and lack of knowledge about host–parasite relationships[4]. Hence, there is a potential to increase sandalwood production through better understanding of the nature of host–parasite relationship, production of high quality planting materials and knowledge of its silviculture. Presently, only a few studies are available on the relation of host in field grown sandal tree. Understanding of haustorial anatomy would give important clues on how sandal takes up food materials from host plants through specialized tissue. Considering the above, studies were carried out to understand the physiological state of haustoria in the field grown sandal tree.

The experiments were carried out in field-established six-year-old sandal trees at the Kerala Agricultural University, Thrissur district, Kerala (10.54°N, 76.28°E). The study compared the number and structure of haustorial connections in sandal with host (*Casuarina*) established in the same pit and sandal growing alone without host (host plant – *Casuarina* dead naturally after establishment of sandal). Two sample trees each were excavated to study the physical association between the sandal tree with the nearby plants including the host. Soil around one quarter area around sandal tree basin was carefully excavated by applying waterjet. The number of functional and non-functional haustoria on host roots was carefully recorded from excavated area.

Anatomical studies were conducted to understand the functional status of sandal–haustoria association. Thin (2–5 μm) microscopic sections of sandal–haustoria were taken following standard procedures of fixing, tissue processing and staining. Functional status of haustoria was studied by observing the movement of radioactive phosphorus (^{32}P) from *Casuarina* to sandal. The translocation through the haustoria from host plant to sandal tree and back was studied by labelling host plant (*Casuarina*) and wild grasses growing around sandal tree with ^{32}P and observing the translocation of radio-label to sandal tree. The first treatment of labelling host plant with ^{32}P was done in two different ways. One by labelling the host plants (*Casuarina*) without sandal tree in the same pit but growing between the rows and the other in the host plants (*Casuarina*) growing with sandal in the same pit. Sandal trees as well as *Casuarina* growing around the labelled plants were traced for ^{32}P. Sandal trees were labelled with ^{32}P to study possible reverse translocations from sandal to host plants.

The diluted ^{32}P sample was applied to the host plant by root feeding. The feeder roots of the host plant were

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excavated and inserted into a polyethene tube of size (2×15 cm^2). ^{32}P solution at the rate of 1.2 mCi in 20 ml was dispensed into the polythene tube, sealed and used for labelling one host (*Casuarina*) plant. Similar study was conducted with sandal grown along with cocoa, cashew, coconut, teak and rubber. For labelling grass species growing around sandal tree, only 0.06 mCi, made up to 1 ml was used. Fresh leaf samples were collected from both host and sandal tree at 1 h, 2 h, 6 h, 2 days, 8 days and 16 days after ^{32}P application and were assessed for^{32}P activity. Radioactivity was determined in a computer-controlled liquid scintillation system (Hidex-Triathler) using Cerenkov counting mode and the activity was expressed as counts per minute (cpm g^{-1}).

The number of functional and non-functional haustoria in host roots is shown in Table 1. For this classification, active translocation of water and nutrients between host and sandal tree was taken as the presence of an active hausteria. Sandal trees with hosts had a larger number of haustoria compared to sandal without host. However, sandal trees without host were also found to have haustorial connections with the roots of adjoining plants. A maximum number of haustorial formation with good host was observed, thus significantly enhancing the growth and nutrient status of sandal seedlings[5]. It was also observed that the tissue graft between host root and sandal haustoria was quite firm and not easily detachable during the excavation process.

Anatomy of sandal–haustoria attached with the host (*Casuarina*) (Figure 1), showed close vascular connections between the sandal tree and the host. This is supported by the observation of tracheary elements within haustoria of six santalaceous root parasites examined by scanning electron microscopy with particular reference to the kind of cells which contain granules[6]. The earlier studies called it ‘phloeotracheids’. It was observed that the granules occur in short vessel elements and in imperforate cells which are thought to represent a specialized type of tracheid. Earlier studies[7] also detected that in haustorium of *Osyrisarborea*, a distinct interrupted zone was present above the vascular core. The majority of xylem elements observed in the vascular core was perforated[7]. It was suggested that these graniferous tracheary elements function in the regulation of pressure and flow of sap[7]. Vascular connectivity between sandal and host root through haustoria was also reported earlier. Intimate vascular connectivity was earlier observed between the roots of the host and sandal tree[8]. The structure functioned as a unified unit serving the physiological requirement of sandal. Although similar observations were reported, direct lumen–lumen xylem connections between the xylem of the host and the parasite were absent (Figure 3). This confirms that the movement of xylem sap from host to sandal occurs principally via pits of host xylem elements.

The counts of ^{32}P in sandal tree, translocated from the host plant at different time intervals, after labelling the host plant with ^{32}P are shown in (Figure 3). During the initial two hours, after labelling the host with ^{32}P, no notable counts were observed in sandal tree. However, higher counts were logged after 6 h of labelling the host plant with radio labelled probe. This indicates that the rate of translocation of xylem sap from host to sandal tree is very rapid. The peak count of ^{32}P in sandal tree was observed on eighth day of the labelling, showing that the translocation of ^{32}P continued up to the eighth day. The reduction trend after the eighth day up to sixteenth day may be due to the decay of ^{32}P in both the host and sandal.

Table 1. Number of sandal–haustoria on host root

	Functional haustoria	Non-functional hustoria
Sandal + *Casuarina*	44	6
Sole sandal	12	6

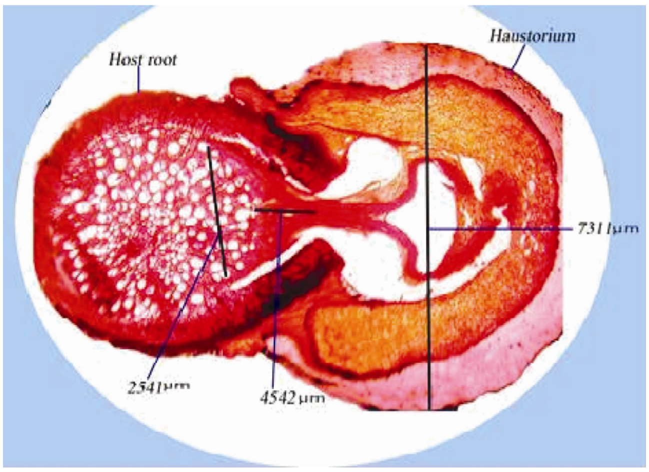

Figure 1. LS of sandal-haustorium with host *Casuarina* (10×).

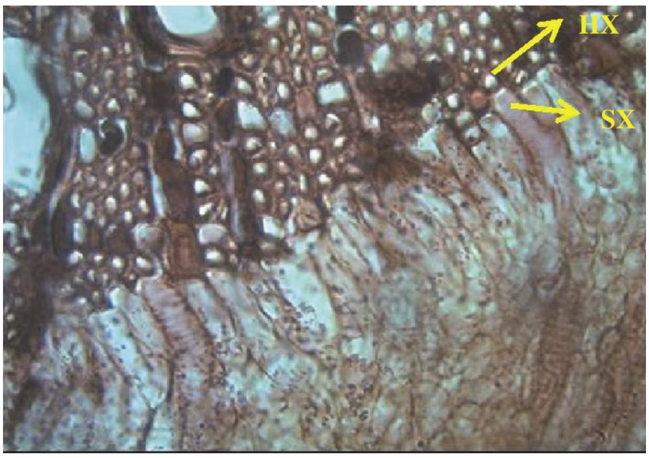

Figure 2. LS of sandalwood haustoria showing xylem–xylem connection between sandalwood and host root (40×). HX, Host xylem; SX, Sandal–haustorial xylem.

Table 2. Translocation of ^{32}P from labelled host to sandal tree at different distances

	^{32}P count (cpm g^{-1})				
Treatments	Labelled *Casuarina*	Sandal in same pit	Sandal at 1.5 m from host	Sandal at 2.5 m from host	Sandal at 3 m from host
C + S*	360	283	216	180	–
C*	263	–	260	248	200

*Indicates ^{32}P labelled plant; C, *Casuarina*; S, Sandal tree.

Table 3. Translocation of ^{32}P from sandal tree to host trees

	^{32}P counts (cpm g^{-1})		
Treatments	Sandal	*Casuarina***	Teak**
Sandal + *Casuarina* + Teak*	513	183	275

*Indicates ^{32}P labelled plant. **All the hosts were planted in the same pit as that of sandal tree.

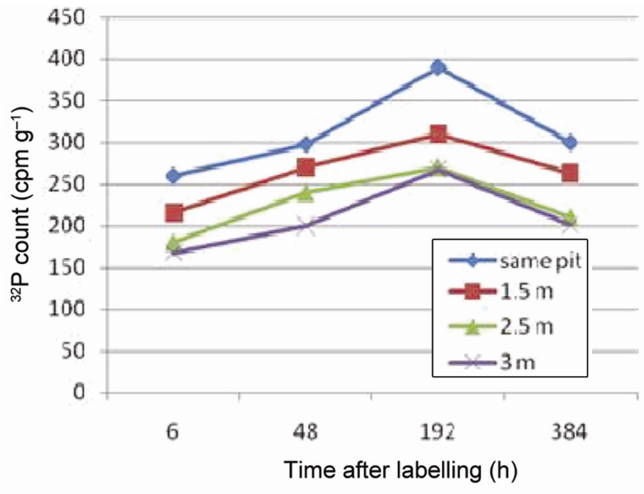

Figure 3. Count of ^{32}P translocated from host, *Casuraina* to sandal trees growing at different distances on different time intervals.

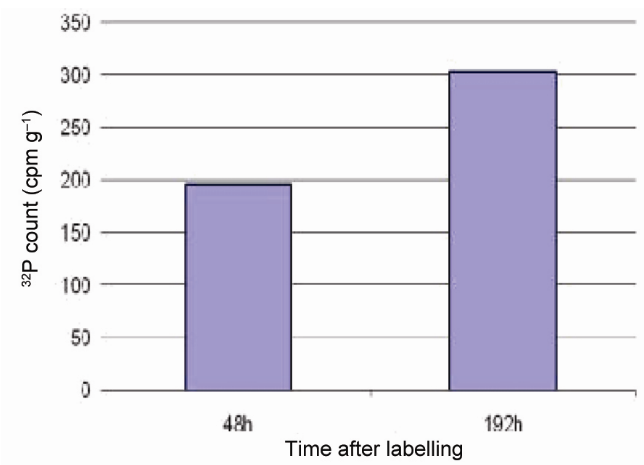

Figure 4. Count of translocated ^{32}P from wild grass to sandal tree.

Translocation of radio-labelled phosphorus from the host to sandal tree growing at different distances is shown in Table 2. The ^{32}P in labelled *Casuarina* grown with sandal tree in the same pit was more as compared to *Casuarina* grown alone. Sandal tree in the same pit with *Casuarina* showed a ^{32}P count of 283 cpm g^{-1}. It was further noticed that a sandal at a distance of 1.5 m away from this treatment had a ^{32}P count of 216 cpm g^{-1}. Similarly, it was also observed that sandal situated at a distance 1.5 m away from a radio labelled *Casuarina* standing alone had a ^{32}P count of 260 cpm g^{-1}. Interestingly, it was observed that the labelled *Casuarina* and sandal had nearly the same ^{32}P count (260 cpm g^{-1} and 263 cpm g^{-1}). The ^{32}P count in sandal tree which was 2.5 m and 3 m away from labelled *Casuarina* also showed appreciable count (248 cpm g^{-1} and 200 cpm g^{-1} respectively).

Casuarina being a drought adapted species with needles in place of leaves, dilution effect is less and hence most of the ^{32}P absorbed was translocated to sandal tree. The ^{32}P count in sandal tree translocated from wild grass growing around the sandal tree is shown in Figure 4. There was significant transfer of ^{32}P count (200–300 cpm g^{-1}) from the labelled wild grass to sandal tree. The ^{32}P from hosts were translocated to sandal tree in both cases. Interestingly, indications of reverse translocation from sandal tree to host plant were evident from the data on ^{32}P count translocated from labelled sandal tree to host plants (Table 3). The translocation from sandal tree to host *Casuarina* was 26% and to teak was 34.89%. From the data, it is evident that translocation from sandal tree to host is also equally efficient.

Root connections between hosts other than *Casuarina* and sandal in the same pit were also studied (Table 4). The translocation from hosts to sandal varied from 27.6% to 78.5%. The percentage of total ^{32}P count detected in sandal tree and host plant also varied depending on the species of host plant and the number of host species present in the same pit as that of sandalwood tree. Translocation varied from 27.65%, when rubber was host to 71%, when cocoa was the host. The multiple plants present in the same pit as sandalwood tree also showed ^{32}P count translocated from the labelled host plant. As the host plants did not have root connections between them, translocation from labelled host to other host plants in the pit or in the adjacent pit may be mediated through

Table 4. Translocation of ^{32}P from host trees to sandal tree

	^{32}P counts (cpm.g^{-1})					
Treatments	Sandal	Host 1**	Host 2**	Host 3**	Total count of sandal and treated host (cpm g^{-1})	Percentage count in sandal with treated host (%)
Sandal + Cocoa*	251	102			353	71.10
Sandal + Cashew*	320	275			595	53.78
Sandal + Cashew* + *Casuarina*	198	224		170	422	46.91
Sandal + Teak*	542	376			918	59.04
Sandal + Teak* + *Casuarina*	321	479	119		800	40.12
Sandal + Coconut* + *Casuarina*	120	289	126		406	29.55
Sandal + *Casuarina**	458	161			619	73.20
Sandal + Coconut* + *Casuarina* + Rubber	215	527	132	120	742	28.9
Sandal + *Casuarina** + Rubber	483	132	124		615	78.53
Sandal + *Casuarina** + Teak	196	155	316		351	55.84
Sandal + Rubber*	142	372			514	27.62
Sandal + Rubber* + *Casuarina*	217	436	99		653	33.23

*Indicates ^{32}P labelled plant. **All the host plants were planted in the same pit as that of sandalwood tree.

sandalwood tree which might have formed functional haustorial connections in all the host plants surrounding it.

This study indicates that sandal tree can form a network of roots aided by haustoria, between several plants including grass growing around it. It is further observed that the host tree need not be planted in the same pit as that of the sandal tree since sandal roots can form functional haustorial connections with trees as far as 3 m from it. A best field host tree-sandal tree would be that with more functional haustoria, but with minimum competition for above ground resources. Planting sandal tree as an intercrop with suitable planting geometry from main crop should be studied to reduce the impact of parasitic nature of sandal in agro-forestry situations. Properly designed planting will ensure regular periodical returns from crops while also ensuring significant income in long term from sandal.

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